

Applicant: Cy A Stein et al.
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Filed: April 11, 2001
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for bcl-xL protein was performed as described above. Results for bcl-xL protein expression were confirmed by Northern blot analysis for bcl-xL mRNA expression, demonstrating significant elevation of this mRNA in bcl-xL transformed cell lines. For the Northern blot analysis, the total RNA was isolated from the cells using TRIZOL reagent (GIBCO BRL), and 20 µg aliquotes were separated in RNA-formaldehyde gel, blotted onto nylon membranes (Schleicher & Schull), UV-linked and prehybridized for two hours at 42 °C in the standard hybridization solution. Then the blot was hybridized overnight with the PCR-amplified fragment of human bcl-xL cDNA at 42 °C. Bcl-xL coding fragment was amplified from pSFFV/bcl-xL plasmid using bcl-x specific primers. The primer sequences were: bcl-x-upstream, 5'-ATGTCTCAGAGCAACCGGGA-3' (SEQ ID NO:36); and bcl-x-downstream, 5'-TCATTCCGACTGAAGAGTG-3' (SEQ ID NO:37). Twenty five cycles of amplification were performed in DNA Thermal Cycler (Perkin-Elmer, Norwalk, CT) at 94 °C (30 sec), 55 °C (30 sec), and 72 °C (30 sec). The PCR products were analysed on a 1.2% agarose gel. The resultant fragment was labeled by random primer method to the specific activity 10⁷ cpm/ng of the probe and used for the hybridization. After washings blots were autoradiographed for 24h at -80 °C. Blots were stripped of radioactivity and reprobed with a ³²P-labeled G3PDH probe to confirm the equal loading.--

A3
cont

In the Sequence Listing:

Please insert the enclosed Sequence Listing (**Exhibit D**) into the above-identified application.

Remarks

The Notice to Comply With Requirements for Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures indicates that the Application clearly fails to comply with 37 C.F.R. §1.821. Applicants attach hereto a copy of the

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Notice as **Exhibit A**. In response applicants submit as **Exhibit B** amended Figure descriptions and specification paragraphs that comply with 37 C.F.R. §1.821. A marked-up copy of the amendments is enclosed as **Exhibit C**. The amendments merely insert sequence ID numbers into the application. Applicants maintain that the amendments to the Figure description and specification raise no issue of new matter. Accordingly, applicants respectfully request that this amendment be entered.

The Notice to Comply With Requirements for Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures indicates that the Application clearly fails to require with the requirements of 37 C.F.R. §1.821 - 1.825. In response, applicants submit as **Exhibit D** hereto a paper copy of the Sequence Listing, a C.R.F Sequence Listing as **Exhibit E**, and a Statement in accordance with 37 C.F.R. §1.821(f) as **Exhibit F**.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicants' undersigned attorney invite the Examiner to telephone him at the number provided below.

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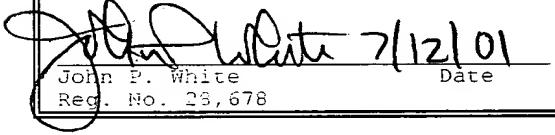
No fee is deemed necessary in connection with the filing of this Communication. If any other fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 03-3125.

Respectfully submitted,



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I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231.


John P. White
Reg. No. 28,678

Date

7/12/01



Mark-up Version to Show Changes Made

In the Brief Description of Figures:

Figure description starting page 5, line 2 has been amended as follows:

--Figure 1

Oligonucleotide sequences complementary to bcl-X_L mRNA; A (SEQ ID NO:1), B (SEQ ID NO:2), C (SEQ ID NO:3), D (SEQ ID NO:4), E (SEQ ID NO:5), F (SEQ ID NO:6), G (SEQ ID NO:7), H (SEQ ID NO:8), I (SEQ ID NO:9), J (SEQ ID NO:10), K (SEQ ID NO:11), L (SEQ ID NO:12), M (SEQ ID NO:13). --

Figure description starting page 5, line 5 has been amended as follows:

--Figures 2A and 2B

Oligonucleotide sequences and analogs thereof complementary to bcl-X_L mRNA; Figure 2A shows: A (SEQ ID NO:14), A' (SEQ ID NO:15), B (SEQ ID NO:16), C (SEQ ID NO:17), C' (SEQ ID NO:18), D (SEQ ID NO:19), E (SEQ ID NO:20), E' (SEQ ID NO:21), F (SEQ ID NO:22), G (SEQ ID NO:23), G' (SEQ ID NO:24); Figure 2B shows, H (SEQ ID NO:25), H' (SEQ ID NO:26), I (SEQ ID NO:27), I' (SEQ ID NO:28), J (SEQ ID NO:29), K (SEQ ID NO:30), K' (SEQ ID NO:31), L (SEQ ID NO:32), L' (SEQ ID NO:33), M (SEQ ID NO:34), M' (SEQ ID NO:35). --

In the Specification:

Paragraph starting page 22, line 33 has been amended as follows:

--Two clones of LNCaP cells overexpressing bcl-xL protein (1072-4 and 1072-5) have been obtained after transfection of wild type LNCaP cells with the plasmid vector pSFFV/bcl-xL and lipofectin. Also a mock transfectant clone of LNCaP cells carrying neo[®]

resistance gene (1072-3) was used for the control experiments. Clone 1072-4 demonstrates 10-fold overexpression, and clone 1072-5 - 4-fold overexpression of bcl-xL protein. Western blot analysis for bcl-xL protein was performed as described above. Results for bcl-xL protein expression were confirmed by Northern blot analysis for bcl-xL mRNA expression, demonstrating significant elevation of this mRNA in bcl-xL transformed cell lines. For the Northern blot analysis, the total RNA was isolated from the cells using TRIZOL reagent (GIBCO BRL), and 20 μ g aliquotes were separated in RNA-formaldehyde gel, blotted onto nylon membranes (Schleicher & Schull), UV-linked and prehybridized for two hours at 42 °C in the standard hybridization solution. Then the blot was hybridized overnight with the PCR-amplified fragment of human bcl-xL cDNA at 42 °C. Bcl-xL coding fragment was amplified from pSFFV/bcl-xL plasmid using bcl-x specific primers. The primer sequences were: bcl-x-upstream, 5'-ATGTCTCAGAGCAACCGGGA-3' (SEQ ID NO:36); and bcl-x-downstream, 5'-TCATTTCCGACTGAAGAGTG-3' (SEQ ID NO:37). Twenty five cycles of amplification were performed in DNA Thermal Cycler (Perkin-Elmer, Norwalk, CT) at 94 °C (30 sec), 55 °C (30 sec), and 72 °C (30 sec). The PCR products were analysed on a 1.2% agarose gel. The resultant fragment was labeled by random primer method to the specific activity 10^7 cpm/ng of the probe and used for the hybridization. After washings blots were autoradiographed for 24h at -80 °C. Blots were stripped of radioactivity and reprobed with a 32 P-labeled G3PDH probe to confirm the equal loading.--